

FIG.  
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#### A. Comparison of disintegrin amino acid sequences:

Contortrostatin DAPANPCDAATCKLITGGSQCADGLCCDQCKFMKEGTVC-RARGDDI-DY-NGISAGG

**Appaglin** EAGEECDGSPENPCCDAATCKLRPGQCAEGLLCCDQCKFMKEGTVC-RARGDDVNDYNGISAGCPRNN

Trigrammin  
EAGEGDCDCGSPANCCDAATCKL<sub>1</sub>PAGAQCGEGLCCDQCSFIEGTVCRIAR<sub>2</sub>GDD<sub>3</sub>DYCNGRSAGGCPRNPFH

Albolabin  
EAGEDCDCGSPANPCCDAACTKL\_PGAQCGEGLCCDQCSFMKGKTCIIRRARGDDLYCNGISAGCPRNPLHA

Elegant in NPCCDATCKLRPGAQCADGLCCDQCRCKKKTCRARGDNDDRCTGQSA  
EAGEECDCGSPE NPCCDATCKLRPGAQCADGLCCDQCRCKKKTCRARGDNDDRCTGQSA  
PRLNGLYS

Kistrin  
GKECDCSSPENPCCDAATCKLRPGAQCGEGLCCEQCKFDRAKGICRIPRGDMDDRCTGQSA  
CPRYH

#### B. Design of PCR primers:

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λgt10  
FORWARD  
PCR-2

DAPANPCCDAATCKL TTGSQCADGLCCDQCKFMKEGTVCRRARGDDL - DY - NGISAG

PCR-1  
λgt10  
REVERSE

### C. Overlapping extension of PCR fragments:

(a) self-extending molecule:

CN-N (approximately 1300 bp)      CN-C (approximately 700 bp)

5' V

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(b) non-self-extensible molecule

CN-N (approximately 1300 bp) CN-C (approximately 700 bp) *kgtr* *reverse*

W. 3' 5'

λgt10 forward  
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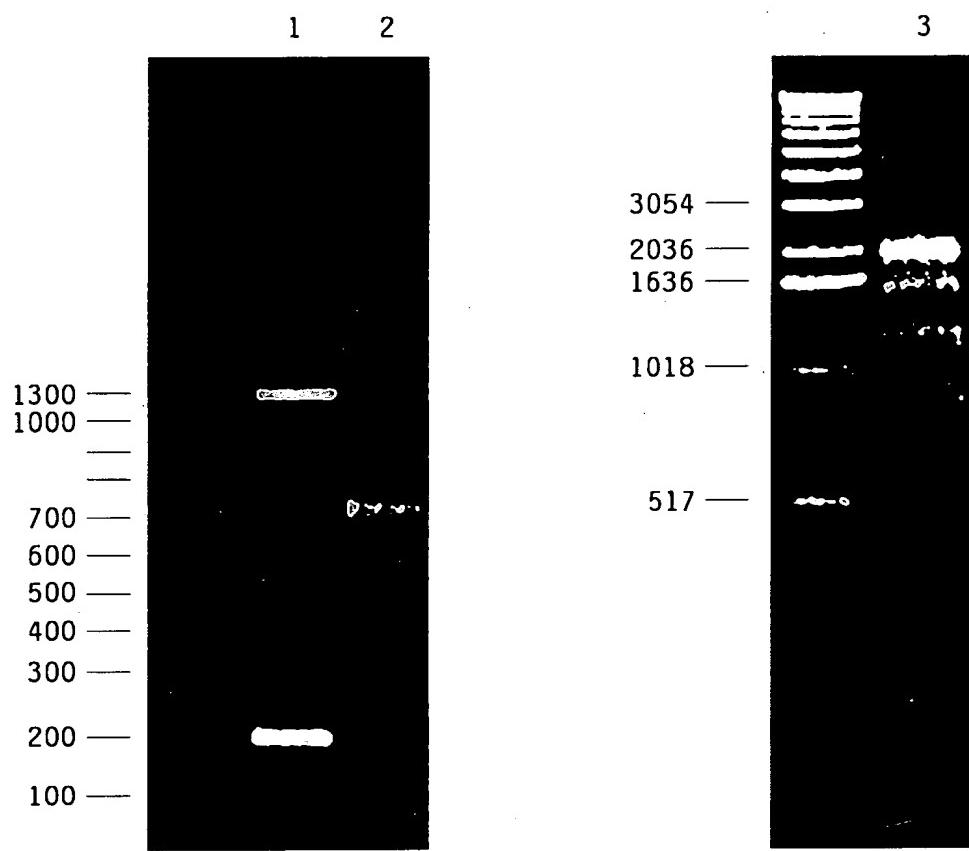


FIG. 2

FIG. 3A

11            20            29            38            47            56  
 5' GA ATT CGG GGT CAA TAG AGG AAG AGC TCA AGT TGG CTT GAA AGC AGG AAG AGA TTG

65            74            83            92            101          110  
 CCT GTC TTC CAG CCA AAT CCA GCC GCC AAA ATG ATC CAG GTT CTC TTG GTC ACT

-----  
 M I Q V L L V T [8]

[1] [119]        128        137        146        155        164  
 CTA TGC TTA GCA GCT TTT CCT TAT CAA GGG AGC TCT ATA ATC CTG GAA TCT GGG

-----  
 L C L A A F P Y Q G S S I I L E S G [26]

[9] [173]        182        191        200        209        218  
 AAT GTT AAT GAT TAT GAA GTA CTG TAT CCA CAA AAA GTC ACT GCA TTG CCC AAA

-----  
 N V N D Y E V L Y P Q K V T A L P K [44]

[27] [227]        236        245        254        263        272  
 GGA GCA GTT CAG CCA AAG TAT GAA GAC ACC ATG CAA TAT GAA TTT AAA GTG AAT

-----  
 G A V Q P K Y E D T M Q Y E F K V N [62]

[45] [281]        290        299        308        317        326  
 GGA GAG CCA GTG GTC CTT CAC CTG GAA AAA AAT AAA GGA CTT TTT TCA AAA GAT

-----  
 G E P V V L H L E K N K G L F S K D [80]

[63] [335]        344        353        362        371        380  
 TAC AGC GAG ACT CAT TAT TCC TCT GAT GGC AGA AAA ATT ACA ACA AAC CCT CCG

-----  
 Y S E T H Y S S D G R K I T T N P P [98]

[81] [389]        398        407        416        425        434  
 GTT GAG GAT CAC TGC TAT TAT CAT GGA CGC ATC CAG AAT GAT GCT GAC TCA ACT

-----  
 V E D H C Y Y H G R I Q N D A D S T [116]

[99] [443]        452        461        470        479        488  
 GCA AGC ATC AGT GCA TGC AAC GGT TTG AAA GGA CAT TTC AAG CTT CAA GGG GAG

-----  
 A S I S A C N G L K G H F K L Q G E [134]

[117] [497]        506        515        524        533        542  
 ACG TAC CTT ATT GAA CCC TTG AAG CTT TCC GAC AGT GAA GCC CAT GCA GTC TAC

-----  
 T Y L I E P L K L S D S E A H A V Y [152]

[135] [551]        560        569        578        587        596  
 AAA TAT GAA AAC GTA GAA AAA GAA GAT GAG GCC CCC AAA ATG TGT GGG GTA ACC

-----  
 K Y E N V E K E D E A P K M C G V T [170]

**FIG. 3B**

605	614	623	632	641	650	
CAG ACT AAT TGG GAA TCA GAT	GAG CCC ATC AAA AAG GCC	TCT CAG TTA AAT CTT				
-----						
[171]Q T N W E S D E P I K K A S Q L N L [188]						
-----						
659	668	677	686	695	704	
ACT CCT GAA CAA CAA GGA TTC CCC CAA AGA TAC ATT GAG CTT GTT GTA GTT GCA						
-----						
[189]T P E Q Q G F P Q R Y I E L V V V A [206]						
-----						
713	722	731	740	749	758	
GAT CAC AGA ATG TTC ACG AAA TAC AAC GGC AAT TTA AAT ACT ATT AGA ATA TGG						
-----						
[207]D H R M F T K Y N G N L N T I R I W [224]						
-----						
767	776	785	794	803	812	
GTA CAT GAA CTT GTC AAC ACT ATG AAT GTG TTT TAC AGA CCT TTG AAT ATT CGT						
-----						
[225]V H E L V N T M N V F Y R P L N I R [242]						
-----						
821	830	839	848	857	866	
GTC TCA CTG ACT GAC CTA GAA GTT TGG TCA GAC CAA GAT TTG ATC AAC GTG CAG						
-----						
[243]V S L T D L E V W S D Q D L I N V Q [260]						
-----						
875	884	893	902	911	920	
CCA GCA GCG GCT GAT ACT TTG GAA GCA TTT GGA GAC TGG AGA GAG ACA GTC TTG						
-----						
[261]P A A A D T L E A F G D W R E T V L [278]						
-----						
929	938	947	956	965	974	
CTG AAT CGC ATA AGT CAT GAT AAT GCT CAG TTA CTC ACG GCC ATT GAG CTT GAT						
-----						
[279]L N R I S H D N A Q L L T A I E L D [296]						
-----						
983	992	1001	1010	1019	1028	
GGA GAA ACT ATA GGA TTG GCT AAC AGG GGC ACC ATG TGC GAC CCG AAG CTT TCT						
-----						
[297]G E T I G L A N R G T M C D P K L S [314]						
-----						
1037	1046	1055	1064	1073	1082	
ACA GGA ATT GTT CAG GAT CAT AGT GCA ATA AAT CTT TGG GTT GCA GTT ACA ATG						
-----						
[315]T G I V Q D H S A I N L W V A V T M [332]						
-----						
1091	1100	1109	1118	1127	1136	
GCC CAT GAG ATG GGT CAT AAT CTG GGT ATT AGT CAC GAT GGA AAT CAG TGT CAT						
-----						
[333]A H E M G H N L G I S H D G N Q C H [350]						
-----						
1145	1154	1163	1172	1181	1190	
TGC GAT GCT AAC TCA TGC ATT ATG AGT GAA GAA CTA AGA GAA CAA CTT TCC TTT						
-----						
[351]C D A N S C I M S E E L R E Q L S F [368]						

**FIG. 3C**

1199	1208	1217	1226	1235	1244												
GAG	TTC	AGC	GAT	TGT	AGT	CAG	AAT	CAA	TAT	CAG	ACA	TAT	CTT	ACT	GAT	CAT	AAC
-----																	
[369] E	F	S	D	C	S	Q	N	Q	Y	Q	T	Y	L	T	D	H	N [386]
-----																	
1253	1262	1271	1280	1289	1298												
CCA	CAA	TGC	ATG	CTC	AAT	GAA	CCC	TTG	AGA	ACA	GAT	ATT	GTT	TCA	ACT	CCA	GTT
-----																	
[387] P	Q	C	M	L	N	E	P	L	R	T	D	I	V	S	T	P	V [404]
-----																	
1307	1316	1325	1334	1343	1352												
TCT	GGA	AAT	GAA	CTT	TTG	GAG	ACG	GGA	GAA	GAA	AGT	GAC	TTT	GAC	GCT	CCT	GCA
-----																	
[405] S	G	N	E	L	L	E	T	G	E	E	S	D	F	D	A	P	A [422]
-----																	
1361	1370	1379	1388	1397	1406												
AAT	CCG	TGC	TGC	GAT	GCT	GCA	ACA	TGT	AAA	CTG	ACA	ACA	GGG	TCA	CAG	TGT	GCA
-----																	
[423] N	P	C	C	D	A	A	T	C	K	L	T	T	G	S	Q	C	A [440]
-----																	
1415	1424	1433	1442	1451	1460												
GAT	GGA	CTG	TGT	GAC	CAG	TGC	AAA	TTT	ATG	AAA	GAA	GGA	ACA	GTA	TGC	CGG	
-----																	
[441] D	G	L	C	C	D	Q	C	K	F	M	K	E	G	T	V	C	R [458]
-----																	
1469	1478	1487	1496	1505	1514												
AGA	GCA	AGG	GGT	GAT	GAC	CTG	GAT	GAT	TAC	TGC	AAT	GGC	ATA	TCT	GCT	GGC	TGT
-----																	
[459] R	A	R	G	D	D	L	D	D	Y	C	N	G	I	S	A	G	C [476]
-----																	
1523	1532	1541	1550	1559	1568												
CCC	AGA	AAT	CCC	TTC	CAT	GCC	TAA	CCA	ACA	ATG	GAG	ATG	GAA	TGG	TCT	GCA	GCA
-----																	
[477] P	R	N	P	F	H	A	*	[483]									
-----																	
1577	1586	1595	1604	1613	1622												
ACA	GGC	AGT	GTG	TTG	ATC	TGA	ATA	CAG	CCT	AAT	AAT	CAA	CCT	CTG	GCT	TCT	CTC
-----																	
1631	1640	1649	1658	1667	1676												
AGA	TTT	GAT	CAT	GGA	GAT	CCT	TCT	TCC	AGA	AGG	TTT	CAC	TTC	CCT	CAA	ATC	CAA
-----																	
1685	1694	1703	1712	1721	1730												
AGA	GAC	CCA	TCT	GCC	TGC	ATC	CTA	CTA	GTA	AAT	CAC	CCT	TAG	CTT	CCA	GAT	GGT
-----																	
1739	1748	1757	1766	1775	1784												
ATC	CAA	ATT	CTG	TAA	TAT	TTC	TTC	TCC	ATA	TTT	AAT	CTA	TTT	ACC	TTT	TGC	TGT
-----																	
1793	1802	1811	1820	1829	1838												
AAC	AAA	ACC	TTT	TTC	CTG	TCA	CAA	AGC	TCC	ATG	GGC	ATG	TAC	AGC	TTA	TCT	GCT
-----																	
1847	1856	1865	1874	1883	1892												
GTC	AAG	AAA	AAA	AAT	GGC	CAT	TTT	ACC	GTT	TGC	CAG	TTA	CAA	AGC	ACA	TTT	AAT
-----																	
1901	1910	1919	1928	1937	1946												
GCA	ACA	AGT	TCT	TCC	TTT	TGA	GCT	GAT	GTA	TTC	AAA	GTC	AAT	GCT	TCC	TCT	CCC

# FIG. 3D

1955            1964            1973            1982            1991            2000  
AAA ATT TCA TGC TGG CTT CCC AAG ATG TAG CTG CTT CCG TCA ATA AAC AAA CTA

2009            2018            2027  
TTC TCA TTC AAA AAA AAA AAC CCG AAT TC 3'

# FIG. 4A

## Proprotein domain:

	1	10	20	30	40	50
	*	*	*	*	*	*
CN	MIQVLLVTLCIAFPYQGSSIILESGNVNDYEVLYPQKVTLAPKGAVQPKY					
Trigramin	MIQVLLITICLAVFPYQGSSIILESGNLNDYEVVYPEKVTALPKGAVQQKY					
Cat	MIQVLLVTICLAAFPYQGSSIILESGNVNDYEVIYPRKVTALPKGAVQPKY					
Jararhagin	ATRPKGAVQPKY					
Ht-e	MIQVLLVTICLAAFPYQGSSIILESGNVNDYEVIYPRKVTALPKGAVQPKY	110	120	130	140	150
	*	*	*	*	*	*
CN	DHCYYHGRIQNDA DSTASISACNGLKGHKLQGETYLIEPLKLSDSEAHAV					
Trigramin	DHCYYHGRIENDAD DSTASISACDGLKGHKLQGEMYLIEPLELSDSEAHAV					
Cat	DHCYYHGRIENDAD DSTASISACNGLKGHKLQGEMYLIEPLKLKDSEAHAV					
Jararhagin	DHCYYHGRIENDAD DSTASISACNGLKGYFKLQRETYFIEPLKLKDSEAHAV					
Ht-e	DHCYYHGRIENDAD DSTASISACNGLKGHKLQGEMYLIEPLKLSDSEAHAV					

## Metalloproteinase domain:

	200	210	220	230	240	
	*	*	*	*	*	
CN	EQQGF.PQR YIEL VVVADH RMFT KYNGNLNTIRI WHEL VNT MNV FYRPLN					
Trigramin	EQQR F.PQR YIKLGIFVDH GM TYK SGN SER ITK RVHQ M INN IN MM CR ALN					
Cat	EHQ KYNPFR FVELFLVV DKAM VT KNN GD LDK I KTR MYE IV NTV NEI YRYM Y					
Jararhagin	EQ QRYDPY KYIE FFVV VDQ GTV TKN NGD LDK I KAR M YEL ANIV NEI FRY LY					
Ht-e	EHQ.....RY VEL FIV VD HGM TYK NGD SD KIR QRV HQ MVN IM KES Y TYM Y	290	300	310	320	330
	*	*	*	*	*	*
CN	LTA IEL DGET I GLAN RGT MCD P KLST GIV QDHS AIN L WVA VT MAHE MG HNL					
Trigramin	LTA TIFNG NVIG RA PVGG MCD PKR SVA I VR DHNA I VFV VAV TM THE MG HNL					
Cat	LTA IDL .DRV IGL AYVG SMC HPK RST GII QDY SEI NL VVA VIMA HE MG HNL					
Jararhagin	LTA IDF NGPT I GYAY I G SMC HPK RSV GIV QD YSP IN LV VAVIMA HE MG HNL					
Ht-e	LTSIA FDE QI I GRAY I GGI CDP KRST GVV QDH SEI NL RV AV TM THE LG HNL					

## Disintegrin domain:

	420	430	440	450
	*	*	*	*
CN	ETGE E SDF --- DAO ABO CC DAAT CJ KTT GSQC ADG KCC DQC JFN JE GT VCR			
Trigramin	EAGE DCDC GSPA ... NPCC DAAT CKL I PG AQC GE GLCC DQCS FIE EG TVCR			
Cat	EV GEE CDC GTP ENC QNE CC DAAT CKL KSG SQC G HGD CCE QCK FS K SG TE CR			
Jararhagin	EV GEE CDC GTP ENC QNE CC DAAT CKL KSG SQC G HGD CCE QCK FS K SG TE CR			
Ht-e	EAGIE CDG GSLE ... NPCC YATT CKM RPG S QCA EGL CCD DQ CR FM KK GT VCR			

## C-terminal domain:

	490	500	510	520	530
	*	*	*	*	*
Cat	NGQP CLD NYGY CYNG NCPI MYHQ CYDL FGAD VYE AED SC FERN QK GN YY GY				
Jararhagin	NGQP CLD NYGY CYNG NCPI MYHQ CYAL FGAD VYE AED SC FKDN QK GN YY GY	590	600		
	*	*			
Cat	PGTK CAD GK VCS NG HC DV ATAY *				
Jararhagin	PGTK CAD GK VCS NG HC DV ATAY				

# FIG. 4B

60            70            80            90            100  
\*            \*            \*            \*            \*  
EDTMQYEFKVNGEPVVLHLEKNKGLFSKDYSETHYSSDGRKITTNPPVE  
EDAMQYEFKVNGEPVVLHLEKNKGLFSEDYSEIHSPDGREITAYPSVE  
EDAMQYELKVNGEPVVLHLGKNKGLFSKDYSETHYSPDGREITTYPLVE  
EDAMQYEFKVNGEPVVLHLEKNKGLFSKDYSEIHSPDGREITTYPPVE  
EDTMQYELKVNGEPVVLHLEKNKGLFSKDYSETHYSFDGRKITTNPSVE  
160            170            180            190  
\*            \*            \*            \*  
YKYENVEKEDEAPKMCVGTVQTNWESDEPIKKASQLNLTP  
FKYENVEKEDEPPKMCVGTVQ.NWESYESTKKASQLNVTP  
YKYENVEKEDEALKMCVGTVQ.NWESYEPIKKASQLVVTA  
FKYENVEKEDEAPKMCVGTVQ.NWKSYEPIKKASQLAFTA  
FKLKNVEKEDEAPKMCVGTVQ.NWESYEPIKKASDLNLNP

250            260            270            280  
\*            \*            \*            \*  
IRVSLTDLEVWSDQDLINVQPAAADTLEAFGD.WRETVLLNRISHDNAQL  
IVTTLCSVLEIWSEKDLITVQ.ASAPTTLTLFGAWRETVLLNRTSHDNAQL  
IHALVGLEIWSNEDKITVKPEAGYTLNA.FGEWRKTDLTRKKHDNAQL  
MHVALVGLEIWSNGDKITVKPDVDYTLNS.FAEWRKTDLTRKKHDNAQL  
IDILLAGIEIWSNGDLINVQPASPNTLNS.FGEWRETDLLKRKSHDNAQL  
350            360            370            380            390            400            410  
\*            \*            \*            \*            \*            \*            \*  
GISHDGNQCHCDANSIMSEELREQLSFESDCSQNQYQTYLTDHNPQCMLNEPLRTDIVSTPVSGNELL  
GMHHDEDKCNCN..TCIMSKVLSRQPSKYFSECSKDYYQTFLTNHNPQCILNAPLRTDTVSTPVSGNELL  
GINHDGSGYCGDYACIMRPEISPEPSTFFSNCSYFECWDFIMNHNPECILNEPLGTDIISPPVCGNELL  
GIHHDTGSCSCGDPYCIMGPTISNEPSKFFSNCSYIQCWDFIMNHNPECIINEPLGTDIISPPVCGNELL  
GIHHDTDSCSCGGYSCIMSPVISDEPSKYFSDCSYIQCWEFIMNQKPQCILKKPLRTDTVSTPVSGNELL

460            470            480  
\*            \*            \*  
RARGD.DLDDYCNGISAGCPRNPFH\*A  
IARGD.DLDDYCNGRSAGCPRNPFH  
ASMSECDPAEHCTGQSSEC PADVFHK  
ASMSECDPAEHCTGQSSEC PADVFHK  
VSMVDRN.DDTCTGQSADCPRNGLYG\*

540            550            560            570            580  
\*            \*            \*            \*            \*  
CRKENGNKIPCAPEDVKCGRLYCKDNSPGQNNPCKMFYSNEDEHKGMVL  
CRKENGKKIPCAPEDVKCGRLYCKDNSPGQNNPCKMFYSNDDEHKGMVL

Kistrin

1            10            20            30            40            50            60

•            •            •            •            •            •            •

NH<sub>3</sub>-GKECDCSSPENPCCDAATCKLRPGAQCGEGLCCEQCKFSRAGKICRIP RGD MPDDRCTGQSADCPRYH-COOH

Contortrostatin

1            10            20            30            40            50            60

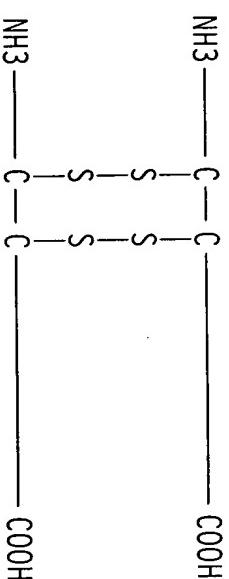
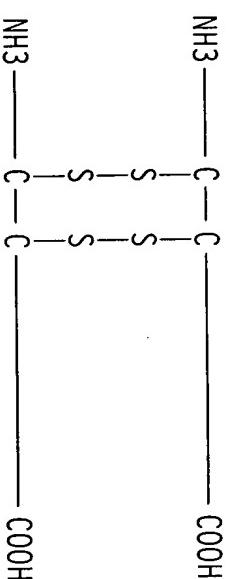
•            •            •            •            •            •            •

NH<sub>3</sub>-DAPANPCCDAATCKLTGSQCADGLCCDQCKFMKEGTVCRR A RGD DLDIYNGISAGCPRNPFHA-COOH

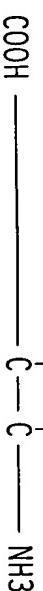


Parallel Model

F/G. 5B

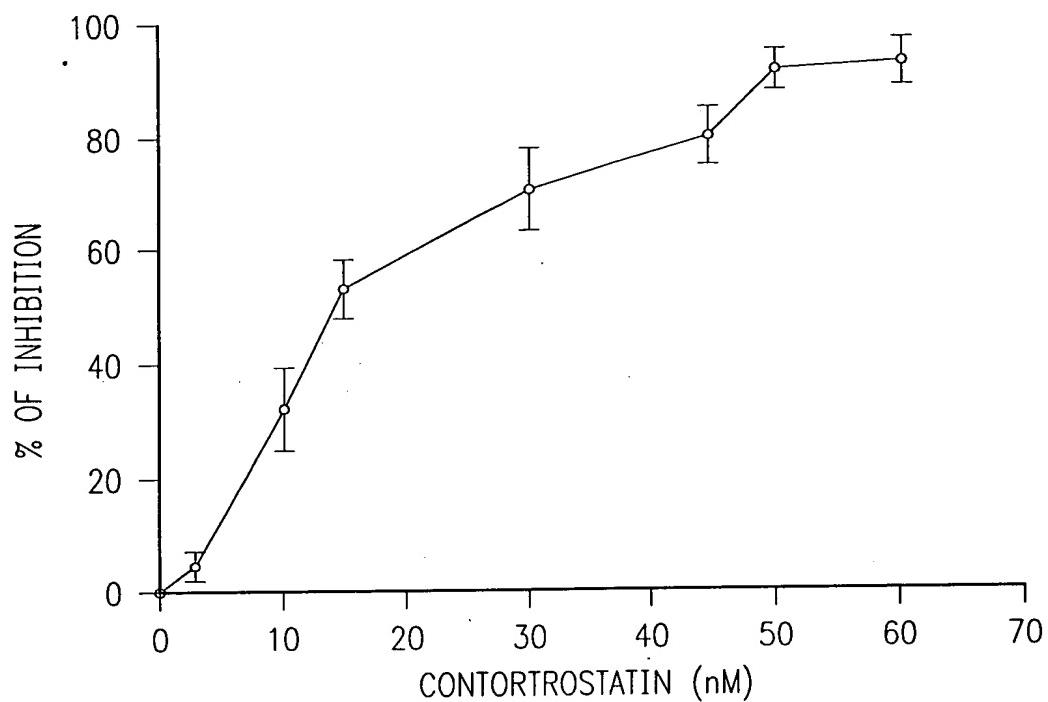


Anti-parallel model

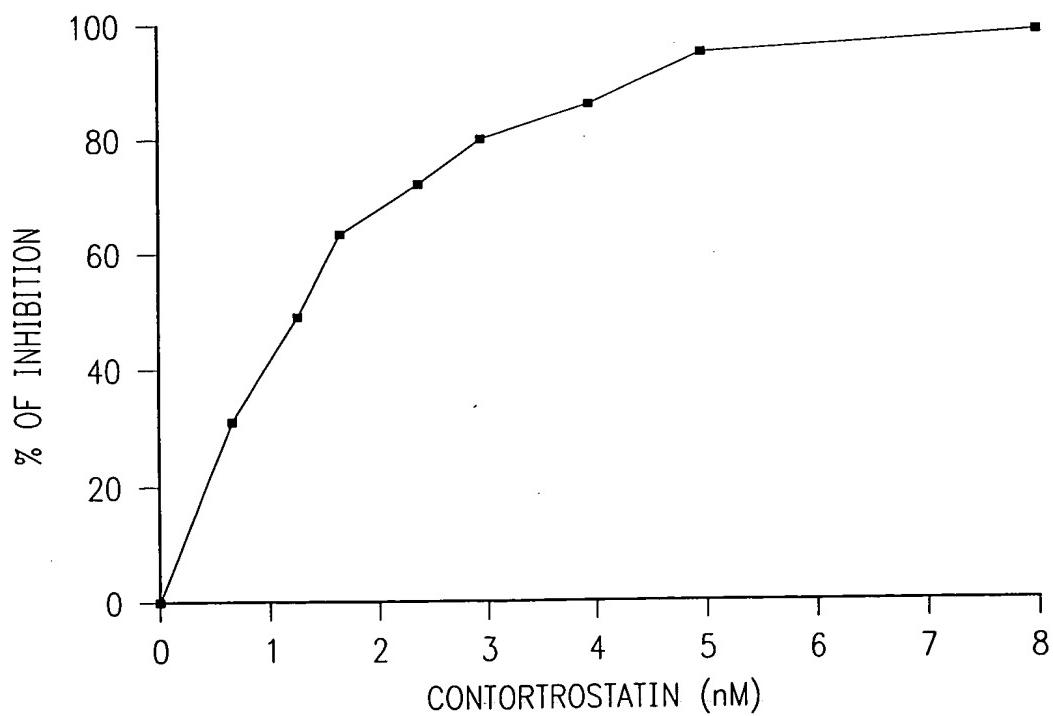


F/G. 5A

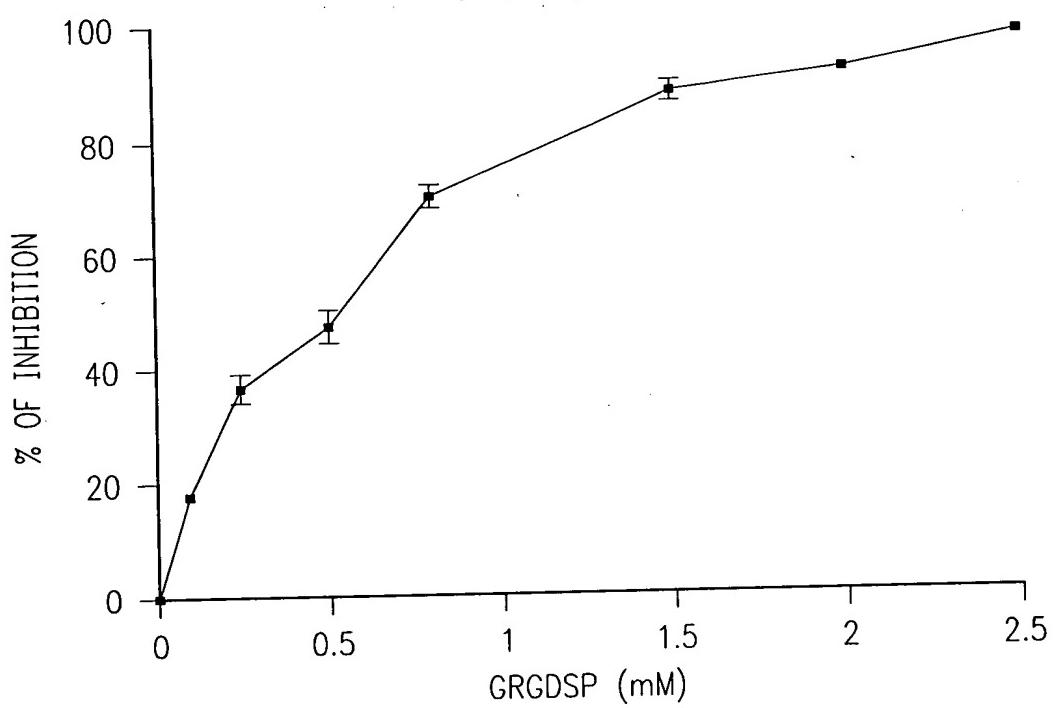
*FIG. 6*



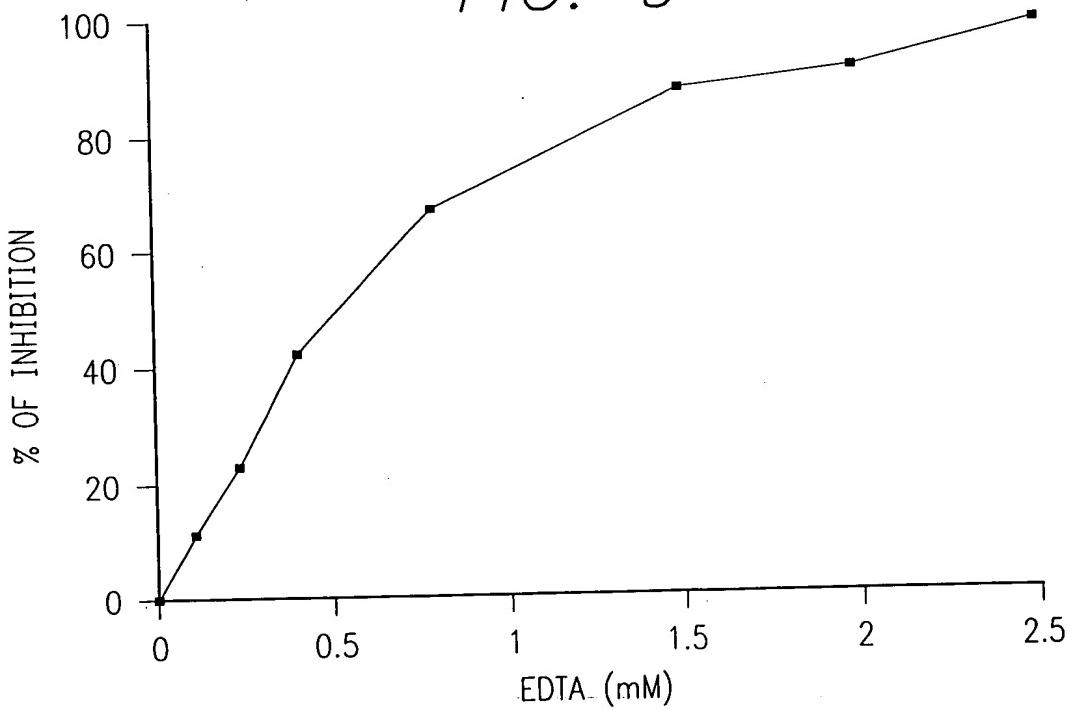
*FIG. 7*

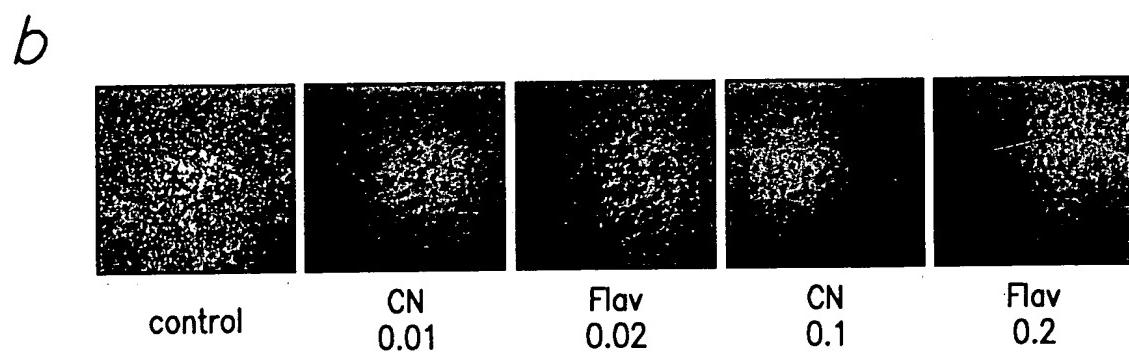
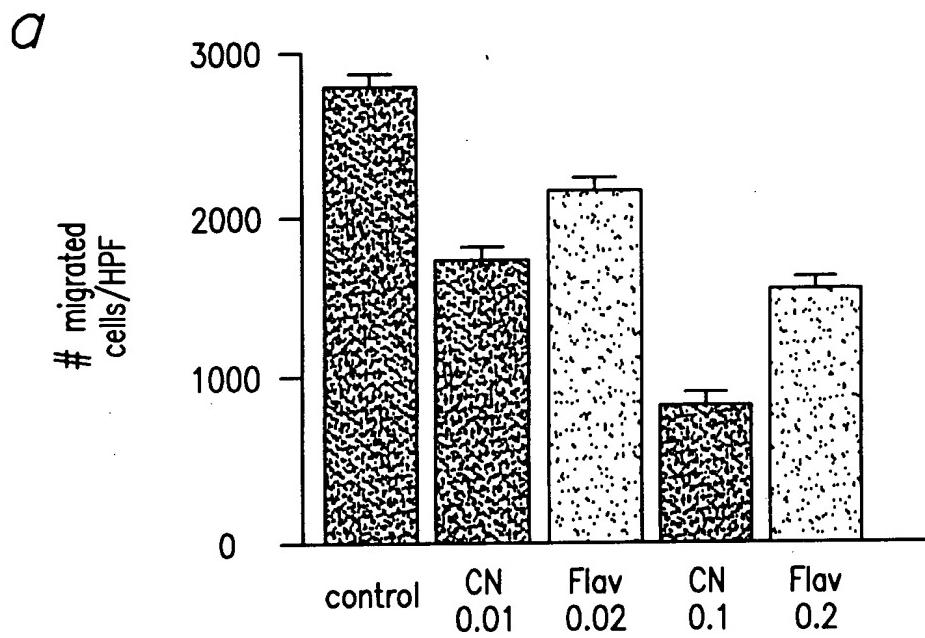


*FIG. 8*



*FIG. 9*





*FIG. 10*

FIG. 11

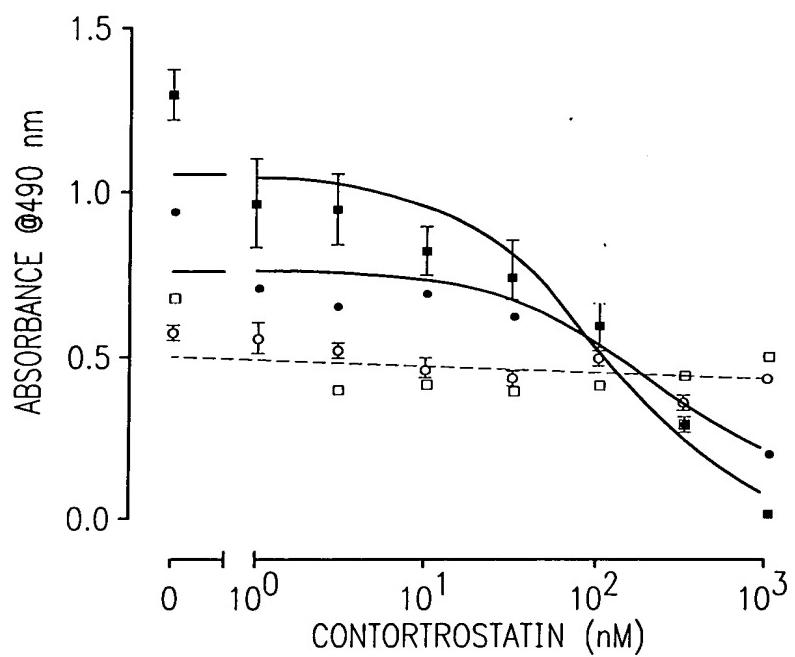
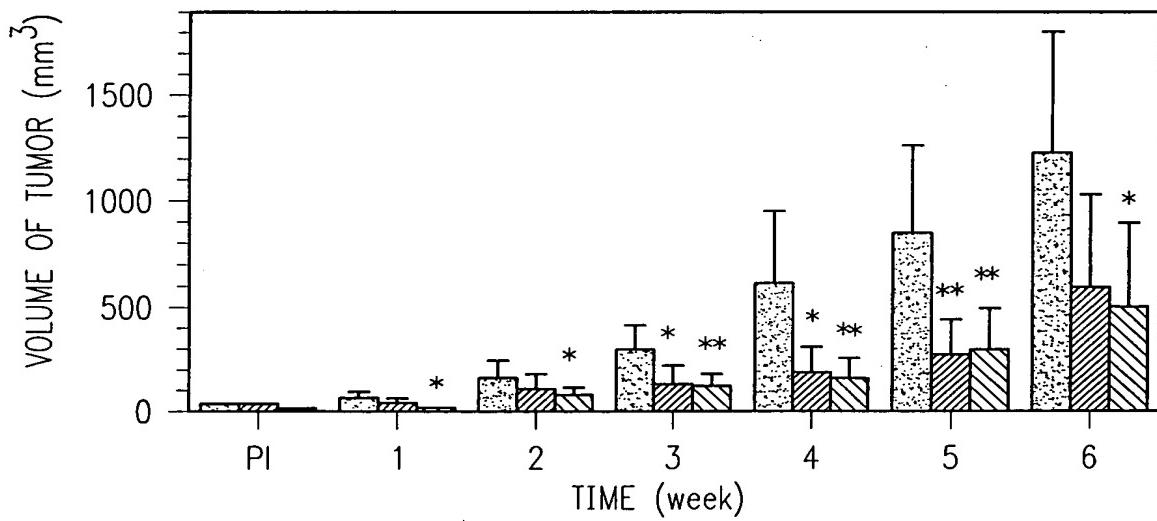


FIG. 12



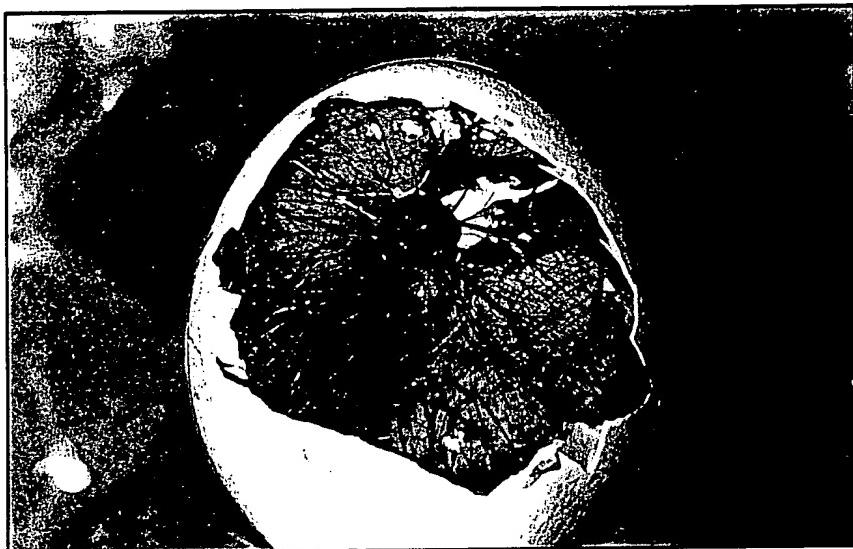


FIG. 13A

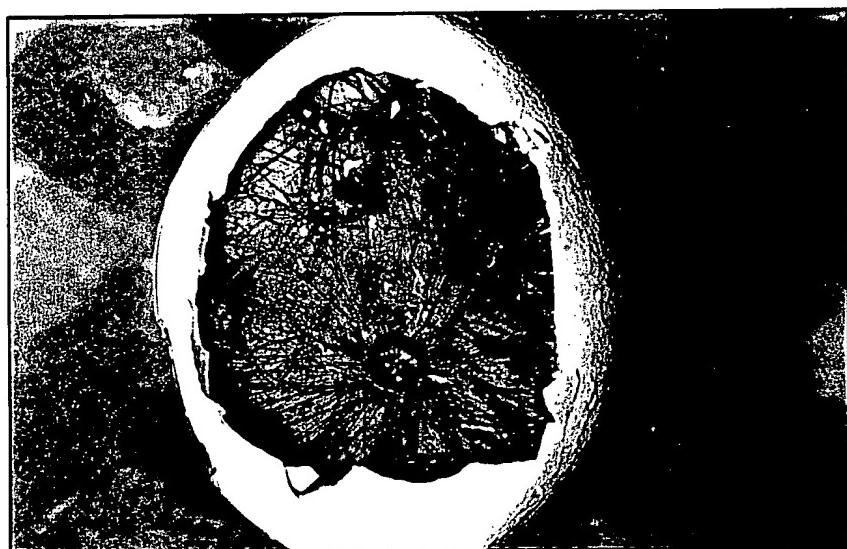


FIG. 13B

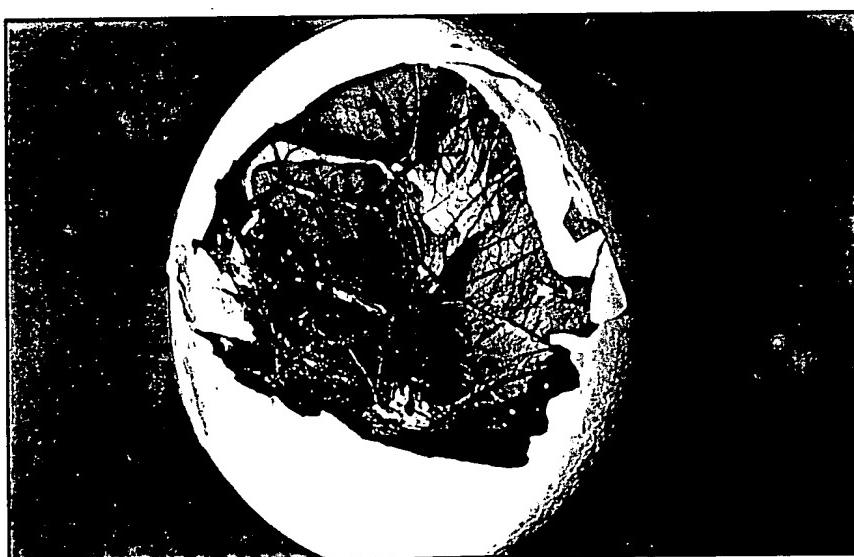
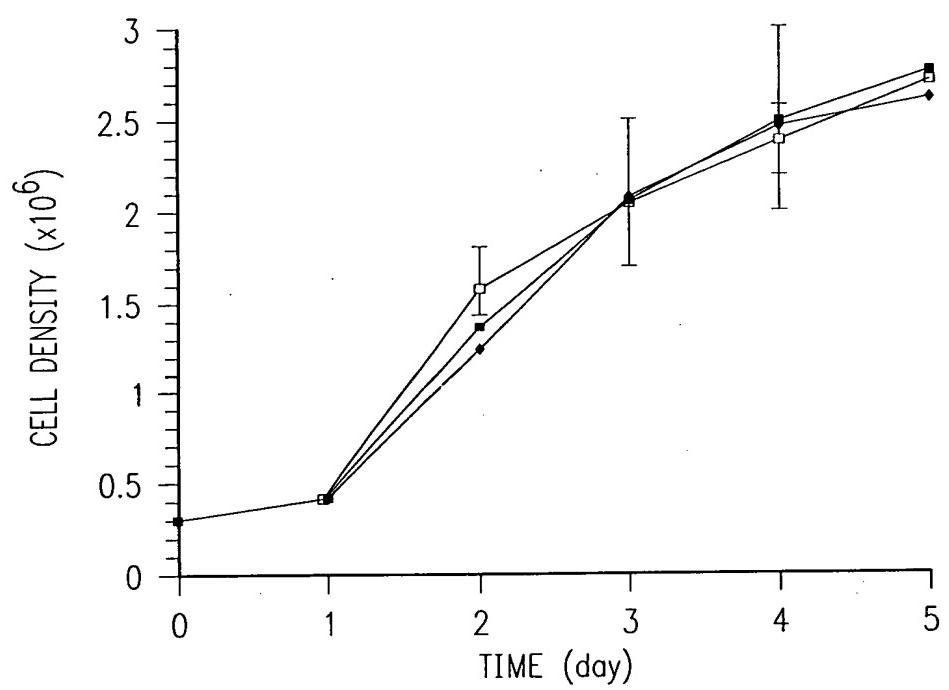
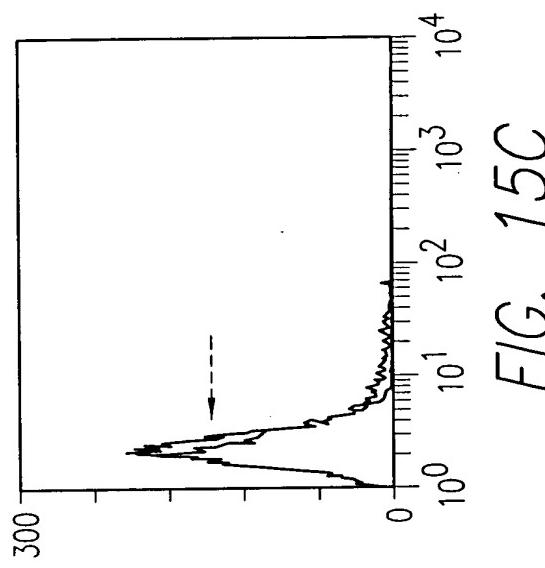
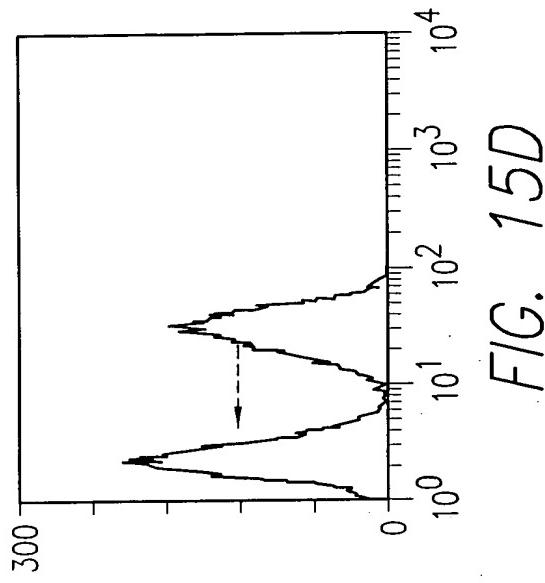
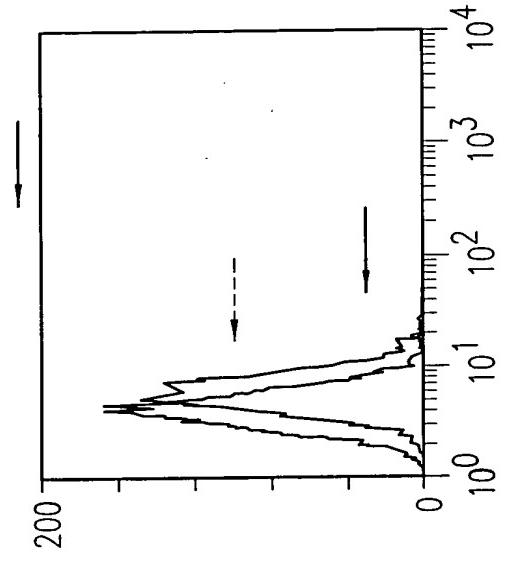
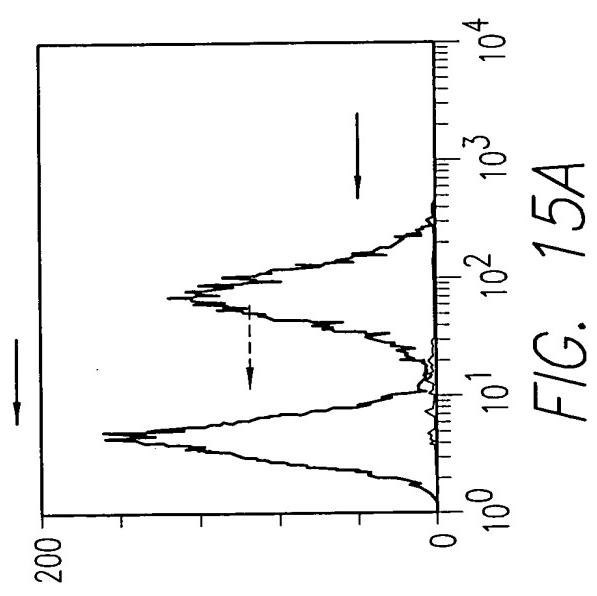


FIG. 13C

FIG. 14





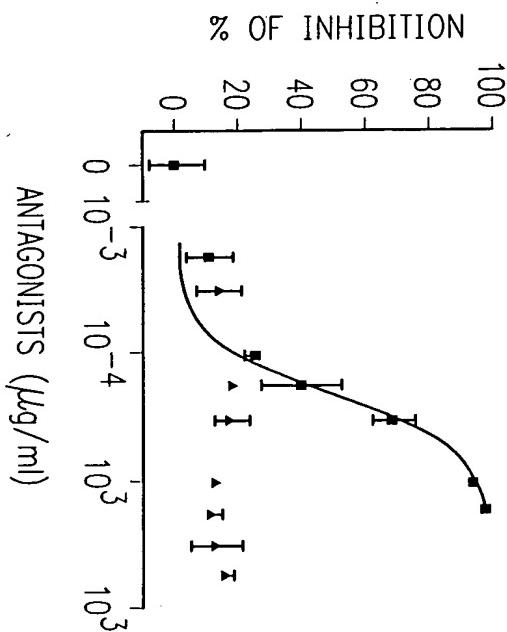


FIG. 16C

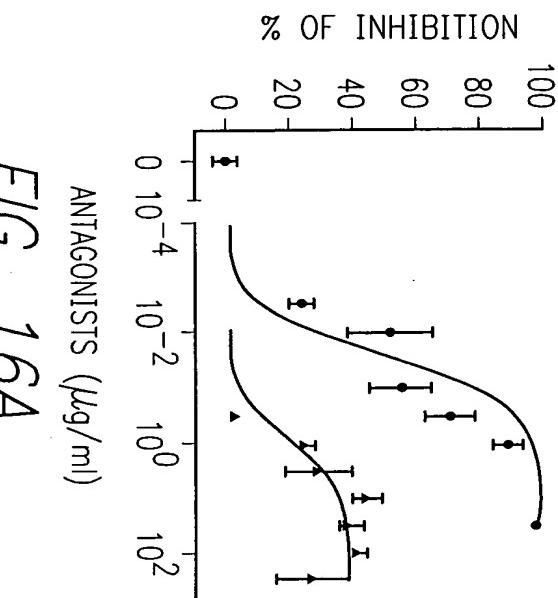


FIG. 16A

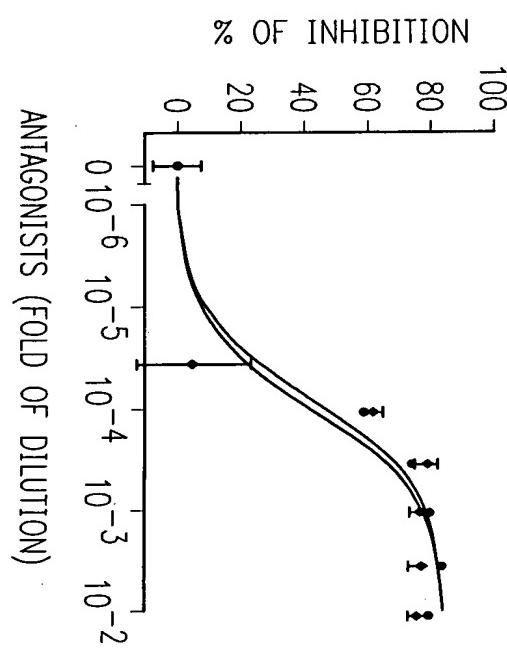


FIG. 16D

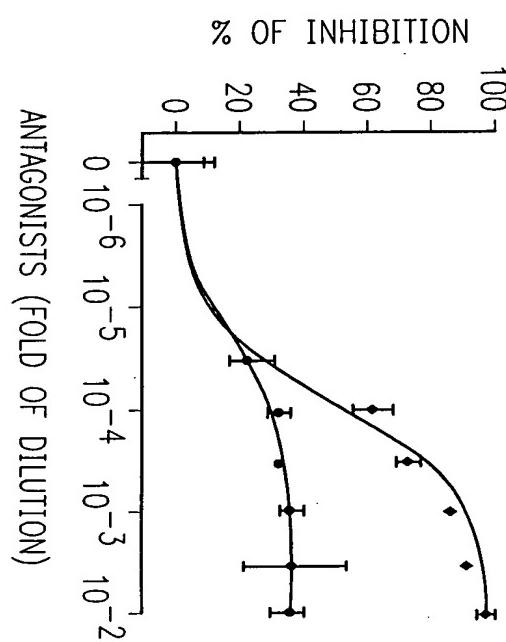


FIG. 16B

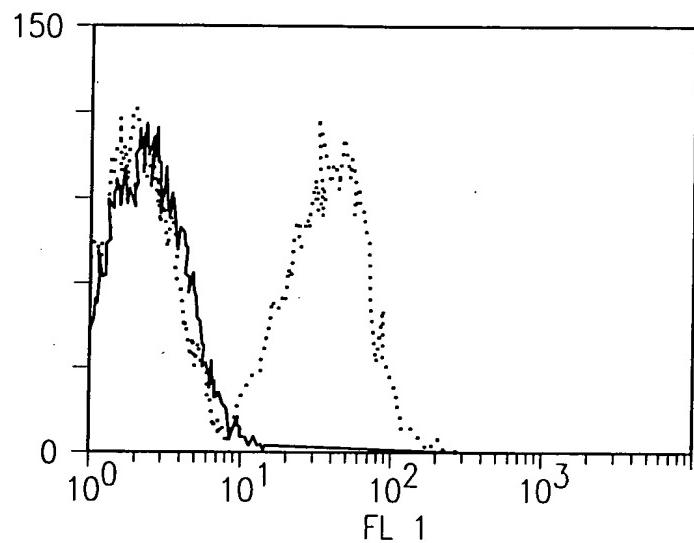


FIG. 17

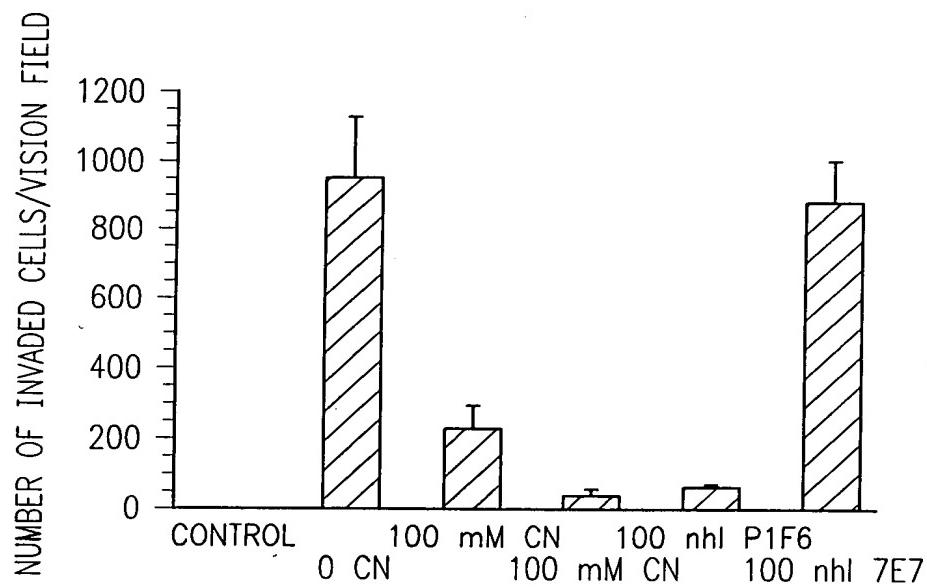


FIG. 18

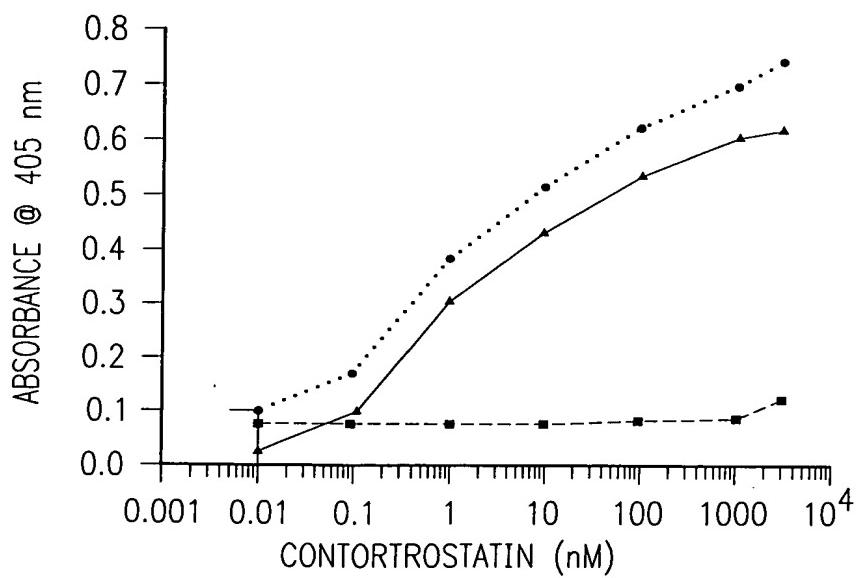


FIG. 19

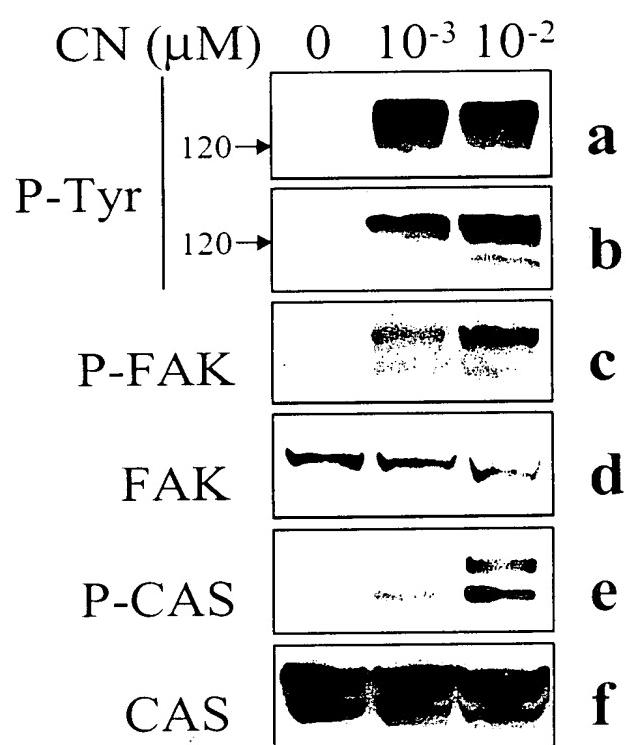


FIG. 20

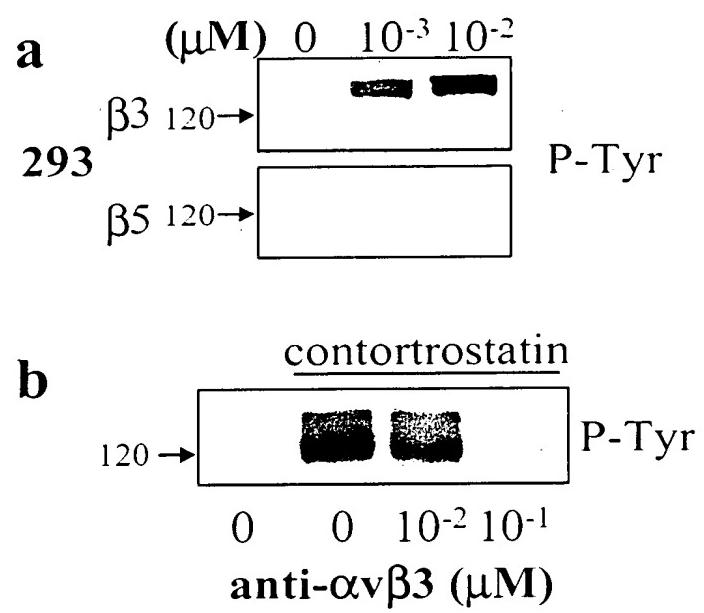


FIG. 21

*FIG. 22*

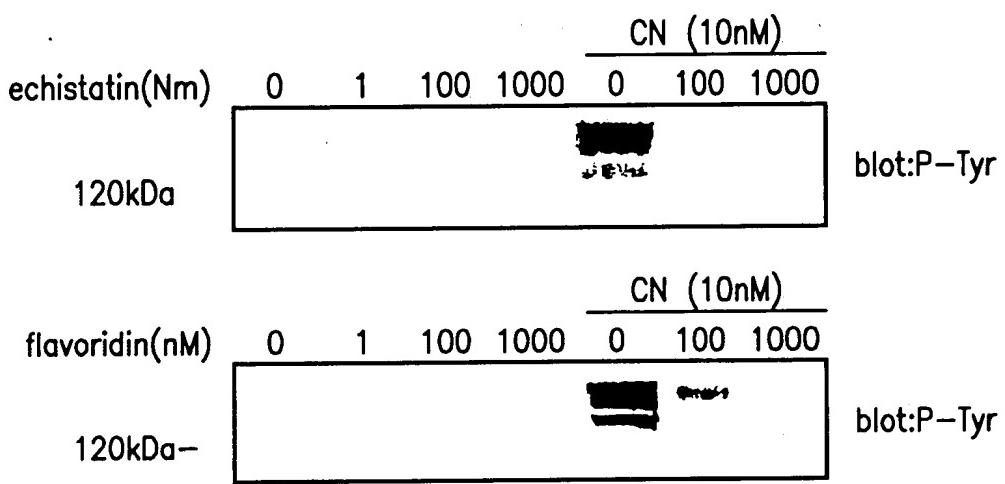


FIG. 23

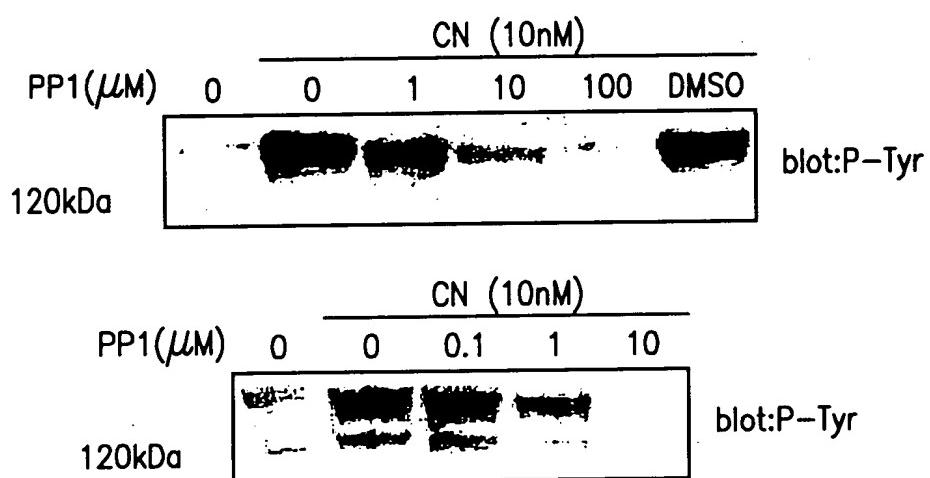
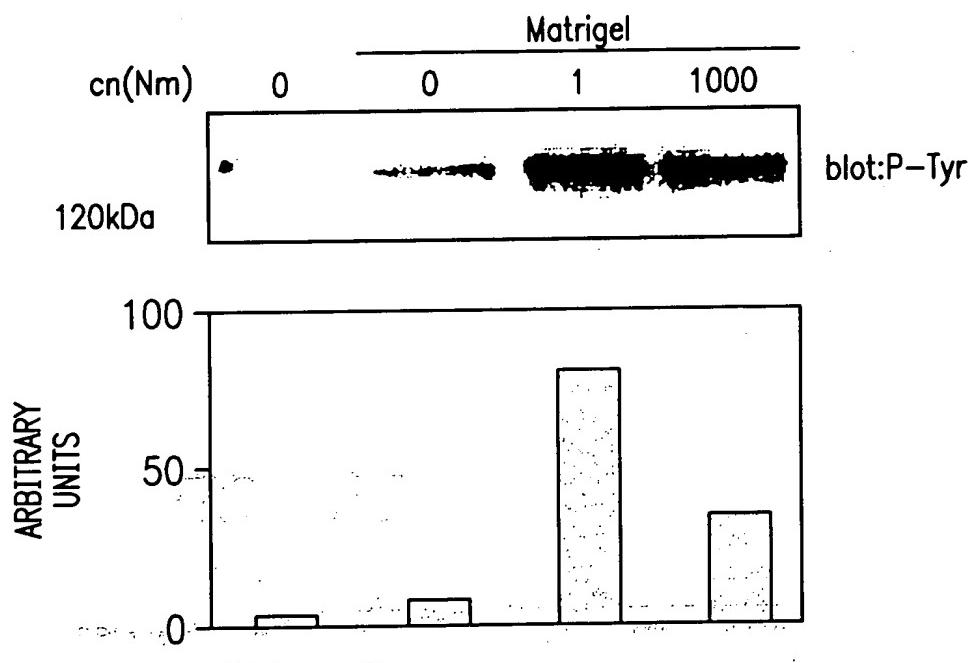


FIG. 24



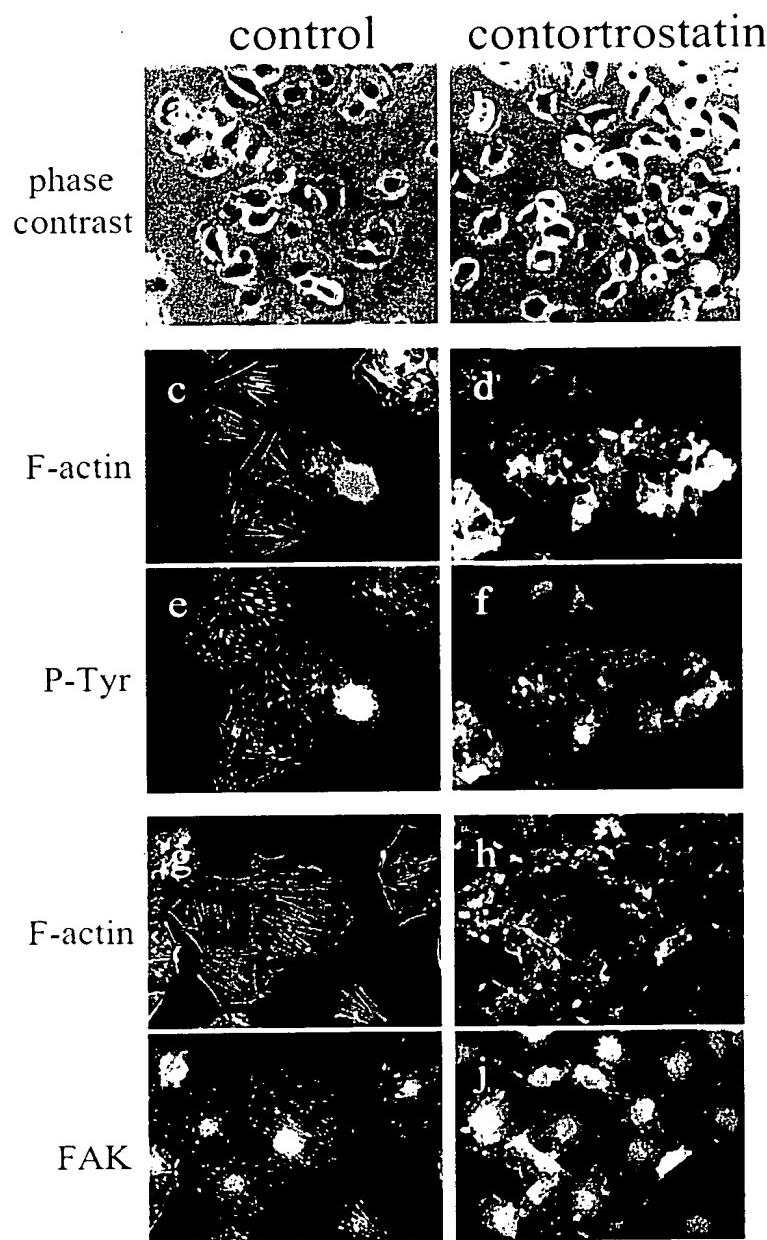


FIG. 25

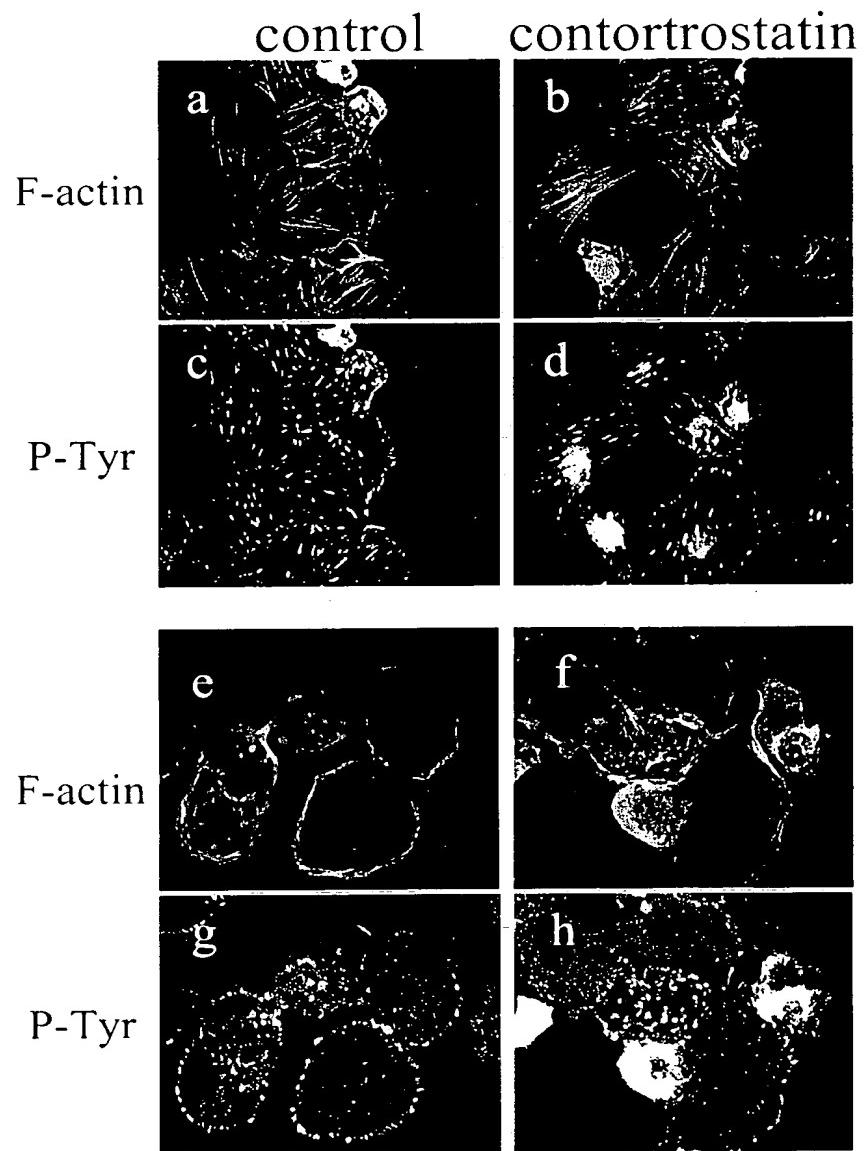


FIG. 26

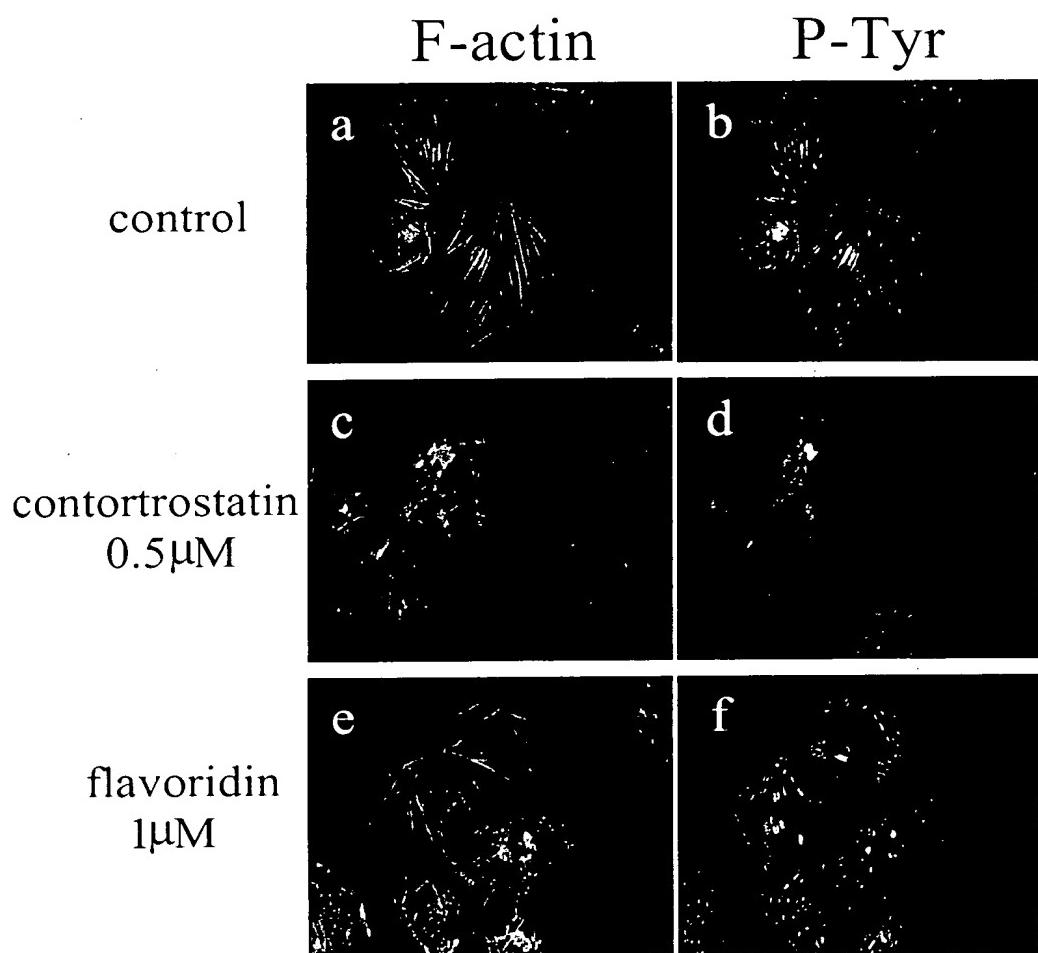
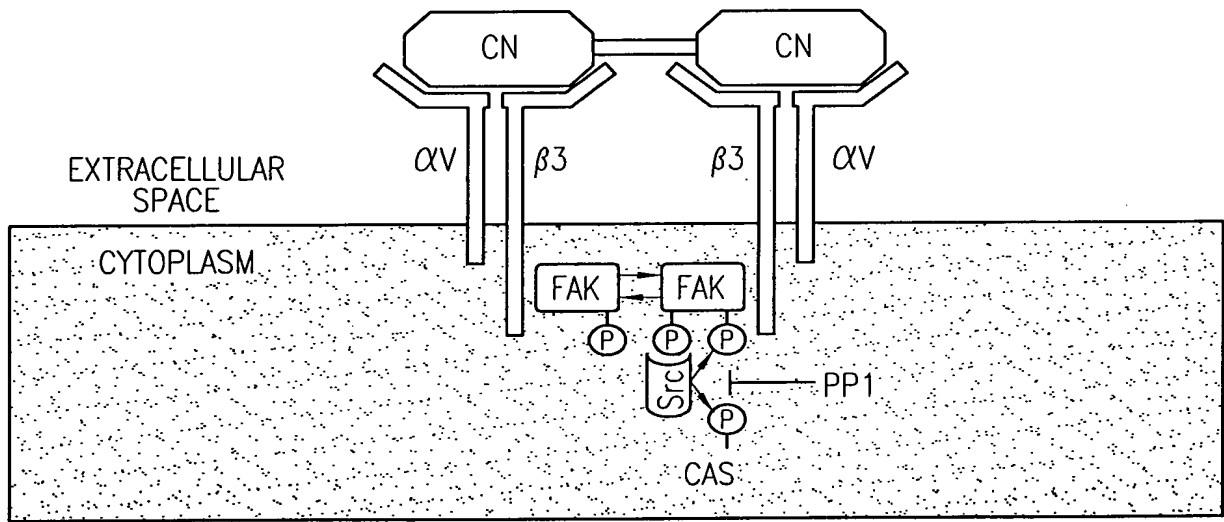


FIG. 27



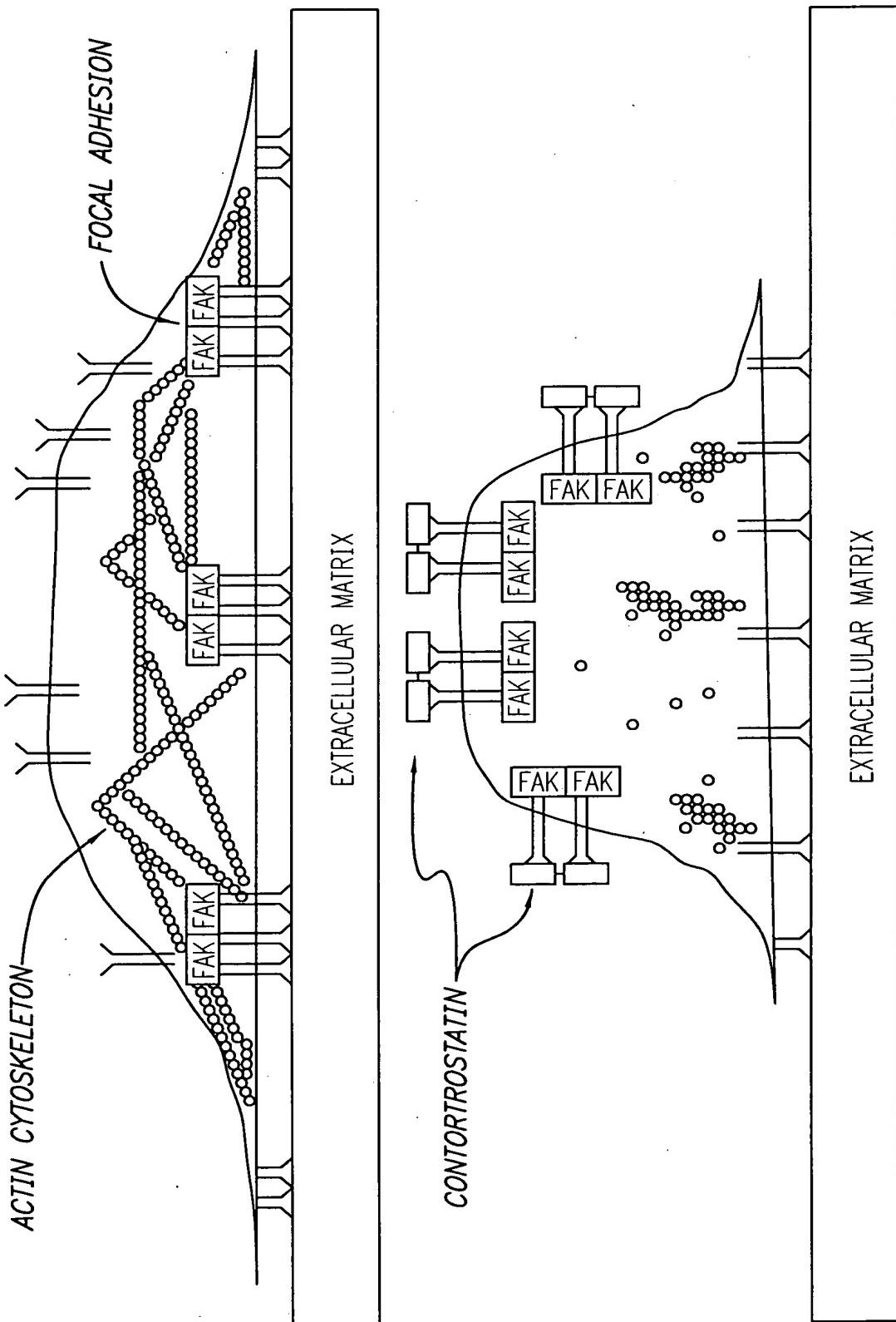


FIG. 29